rinsed in 2X SSC, 0.05% SDS several times at room temperature before being transferred to a wash solution containing 0.1X SSC, 0.1% SDS and agitated at 50°C for 40 minutes. The blots were then covered with plastic wrap, mounted on Whatman paper and exposed to x-ray film at -70°C using an intensifying screen.

REMARKS

I. Status of the Parent Application

The Examiner has asked that page ii of the specification be amended to reflect the statuses of the parent applications. Applicants have so amended the specification in accordance with the Examiner's request.

II. Sequence Identifiers

The Examiner has also objected to the application as failing to comply with the Sequence Listing Rules. In particular, the Examiner has pointed to page 51 and the Brief Description of the Drawings as having no SEQ ID NOS. Applicants have amended the specification to include sequence identifiers where they are appropriate (e.g. page 51, and the Brief Description of the Drawings.)

III. Sequence Disclosure.

To expedite prosecution and comply with 37 C.F.R. §§ 1.821-1.825, Applicants have submitted a substitute sequence listing including two additional sequence disclosures: SEQ ID: 30 and SEQ ID NO: 31.

SEQ ID NO: 30 comprises the amino acid sequence of human DEL-1 and is corresponding to amino acid residues # 33 through # 513 of SEQ ID NO: 14. As illustrated in part below, the first 16 residues of #33 through #513 of SEQ ID NO: 14 match with the first 16 residues starting from

residue #1 of SEQ ID NO: 30 and the last 16 residues of #33 through #513 of SEQ ID NO: 14 match with the last 16 residues ending in residue #481 of SEQ ID NO: 30:

#33 Met Lys Arg Ser Val Ala Val Trp Leu Leu Val Gly Leu Ser Leu Gly .... (SEQ ID NO:14)

#1 Met Lys Arg Ser Val Ala Val Trp Leu Leu Val Gly Leu Ser Leu Gly .... (SEQ ID NO:30)

#498 Gly Arg Ile Thr Leu Ala Ser Glu Leu Leu Gly Cys Thr Glu Glu Glu #513 (SEQ ID NO: 14)

#466 Gly Arg Ile Thr Leu Ala Ser Glu Leu Leu Gly Cys Thr Glu Glu Glu #481 (SEQ ID NO: 30)

SEQ ID NO:31 is the partial amino acid sequence of the human DEL-1 splicing variant, minor form, as shown in FIG 6 and in relevant portions, FIG 11. As illustrated in part below, the first sixteen residues from # 1 through # 16 of SEQ ID NO:31 match with the first sixteen residues in FIG. 11 and the last 16 residues from #88 through # 103 of SEQ ID NO:31 match with the last residues of FIG 11.

#1 Xaa Asp Ile Cys Asp Pro Asn Pro Cys Glu Asn Gly Gly Ile Cys Leu . . (SEQ ID NO: 31)

#1 X D I C D P N P C E N G G I C L ... (FIG. 11)

#88 Ile Asn Glu Cyc Glu Val Glu Pro Cyc Lys Asn Gly Gly Ile Cyc Thr #103 (SEQ ID NO:31)

#88 I N E C E V E P C K N G G I C T #103 (FIG.11)

Applicants respectfully submit that SEQ ID NO:30 is identical to residues #33 through #513 of SEQ ID NO: 14 and SEQ ID NO:31 is identical to residues as shown in FIG. 11. Therefore, no new matter is added in both sequences.

IV. §112, First Paragraph, Rejection

Claims 50-55 and 58-63 were initially rejected under 35 U.S.C. §112, first paragraph, as "containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." In particular, the Office Action stated that no support was found for the claims 50-55 reciting "residues # 33 through 513" and claims 58-63 reciting "residues #33 through #513, with the exception that the amino acid sequence from residues #98 through #107 is removed."

Applicants note that there may be some confusion as to which Sequence Listing Applicants were initially referring to. This confusion arises from the fact that there were two sequence listings submitted in the application, which were different from each other in relevant parts and filed on the same filing date of this application. In the interview granted by the Examiner and conducted on October 9, 2001, the Examiner suggested a substitute sequence listing be submitted that would contain new sequence IDs for the corresponding portion of sequences to which Applicants referred. Applicants have submitted the substitute sequence listing as suggested to overcome the confusion. Since Applicants have cancelled claims 50-55 and 58-63 and submitted new claims 64-84, the rejection has now become moot.

Applicants believe that claims 64-84 are supported by the disclosure in the specification. In the introduction of the application, Applicants state clearly "the invention relates to del-1 nucleotide sequence, Del-1 amino acid sequences, methods of expressing a functional gene product, antibodies specific for the gene product.. . . ." P. 1, ll. 14-16. Applicants then state the need to make antibodies against Del-1. The antibodies can "competitively inhibit activity of Del-1 protein and neutralize its activity. P. 25, ll. 33-34. The antibodies cal also be "used in detecting and quantifying expression of Del-1 levels in cells and tissues such as endothelial cells and certain tumor cells, as well as isolating Del-1 positive cells." P. 25, l. 36 to p. 26, l. 2. Applicants further disclose the method of production of antibodies. "For production of antibodies, various host animal may be immunized by injection with the recombinant or naturally purified Del-1 protein, fusion protein, or peptides." P. 37, ll. 17-19. "Monoclonal antibodies to Del-1 may be prepared by using any technique which provides for production of antibody molecules by continues cells in culture." P. 37, ll. 29-31. "Antibody fragments which contain specific binding sites of Del-1 may be generated by known techniques." P. 38, line 28-29. Finally, the original claim 22 at the time of filing claimed "an antibody which immunospecifically binds to an epitope of the Del-1."

Since antibodies can be produced through immunizing animals by "injection with the recombinant or naturally purified Del-1 protein, fusion protein, or peptides" (P. 37, ll. 17-19) and the peptide sequences of human Del-1 (SEQ ID NO: 30) or the splicing variant Del-1 (SEQ ID NO:31) are disclosed, the making of antibodies that binds to Del-1 can immediately be envisaged.

As a matter of fact, Applicants have disclosed a working example of an antibody that was produced against Del-1. P. 61, ll. 3-33. Accordingly, new claims 64-83 are clearly supported by the disclosure in the specification.

CONCLUSION

As discussed in the Remarks above, Applicants submit that claims 64-84 are presently in condition for allowance and respectfully request a notice to that effect.

Respectfully submitted,

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Clean Set of Claims

64. An antibody which binds to a polypeptide of a mammalian Del-1 or a fragment of the polypeptide of said mammalian Del-1.

65. The antibody of claim 64 wherein the antibody specifically binds to an epitope of said mammalian Del-1.

66. The antibody of claim 64 wherein the antibody is selected from a group consisting of single chain, chimeric, Fab, $F(ab')_2$, and a fragment produced by an Fab expression library.

67. The antibody of claim 64 wherein the antibody is a polyclonal antibody.

68. The antibody of claim 64 wherein the antibody is a monoclonal antibody.

69. The antibody of claim 64 wherein said mammalian Del-1 is a human Del-1.

70. The antibody of claim 69, wherein said human Del-1 comprises the amino acid sequence as shown in SEQ ID NO 30.

71. The antibody of claim 70 wherein the antibody specifically binds to an epitope of the polypeptide of said human Del-1.

72. The antibody of claim 69, wherein said human Del-1 comprises the amino acid sequence as shown in SEQ ID NO 31.

73. The antibody of claim 72 wherein the antibody specifically binds to an epitope of said human Del-1.

74. The antibody of claim 69 wherein the antibody is selected from a group consisting of single chain, chimeric, Fab, $F(ab')_2$, and a fragment produced by an Fab expression library.

75. The antibody of claim 69 which is a polyclonal antibody.

76. The antibody of claim 69 which is a monoclonal antibody.

77. A method of using the antibody in claim 69 wherein the antibody is used to competitively inhibit the binding of said human Del-1 to alpha V beta 3.

78. A method of using the antibody in claim 69 wherein the antibody is used to inhibit the activity of said human Del-1.

79. A method of using the antibody in claim 69 wherein the antibody is used to isolate a cell expressing said human Del-1.

80. A method of using the antibody in claim 69 wherein the antibody is used to detect a cell expressing said human Del-1.

80. A method of using the antibody in claim 69 wherein the antibody is used to detect a cell expressing said human Del-1.

81. A method of using the antibody in claim 69 wherein the antibody is used to competitively inhibit the binding of a molecule to said human Del-1.

82. A method of using the antibody in claim 69 wherein the antibody is linked to a cytotoxic agent.

83. A method of using the antibody in claim 69 wherein the antibody is linked to a radioisotope.

84. A method of using the antibody in claim 69 wherein the antibody is anchored on a solid support.

Clean Set of Amended Specification

1. On page ii, lines 27-31, please delete the paragraph on priority information and insert the following in its place:

This is a divisional application of United States Patent Application Serial NO. 08/659,235, filed June 5, 1996 , now U.S. Patent No. 5,877,281, issued on March 2, 1999, which is a continuation-in-part of United States Patent Application Serial No. 08/480,229, filed June 7, 1995, now U.S. Patent No. 5,874,562, issued on February 23, 1999, each of which is incorporated herein in its entirety.

2. On page 2, last paragraph, (l. 34 spilling over to p. 3, l. 12), please delete this paragraph and insert the following in its place:

Studies from a number of laboratories have characterized the ability of the endothelial cell to dramatically alter basic activities in response to cytokines such as tumor necrosis factor (TNF) -alpha. TNF-alpha stimulation induces significant alterations in the production of vasoactive compounds such as nitric oxide and endothelin, increases surface stickiness toward various types of leukocytes, and modulates the expression of both pro- and anti-coagulant factors (Cotran et al., 1990, *J. Am. Soc. Nephrol.* 1:225-235; Mantovani et al., 1992, *FASEB J.* 6:2591-2599). In turn, endothelial cells have been shown to be an important source for the production of cytokines and hormones, including interleukin 1, 6 and 8 (Gimbrone et al., 1989, *Science* 246:1601-1603; Locksley et al. 1987, *J. Immunol.* 139:1891-1895; Loppnow *et al.*, 1989, *Lymphokine. Res.* 8:293-299; Warner et al., 1987, *J. Immunol.* 139:1911-1917).

3. On page 9, ll. 8-31, please delete the brief descriptions for "Figure 2," "Figure 3A-3E," and "Figure 4A-4C" and insert in their places the following:

Figure 2. Homology analysis between the deduced amino acid sequence of the putative *del-1* gene (m-del1) (SEQ ID NO: 1) and other proteins with "discoidin-like domains." Identical residues are boxed, conserved -residues are shaded (Geneworks, Intelligenetics, Mountain View, CA). m-*del-1* sequence (SEQ ID NO: 1) was derived from a trapped exon and mouse embryo cDNAs. Abbreviations:h-MFG, human milk fat globule protein (SEQ ID NO: 2); h-FV, human coagulation factor V (SEQ ID NO: 3); m-FVIII, mouse coagulation factor VIII (SEQ ID NO: 4); X-A5b1 (SEQ ID NO: 5) and X-A5b2 (SEQ ID NO: 6), b1 and b2 domains of Xenopus neuronal antigen A5; dis-I, discoidin I (SEQ ID NO: 7); consensus sequence (SEQ ID NO: 8).

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Figure 3A-3D. Nucleotide sequence and deduced amino acid sequence of murine *del-1* cDNA (SEQ ID NO: 9) and (SEQ ID NO: 10).

Figure 4A-4C. Nucleotide sequence and deduced amino acid sequence of sequence of human *del-1* cDNA (SEQ ID NO: 11) and (SEQ ID NO: 14).

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4. On page 10, ll. 1-6, please delete the brief description for "Figure 6" and insert in its place the following:

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Figure 6. Amino acid sequence comparison between murine (m-*del-1*) (SEQ

ID NO: 10) and human (h-*del-1*) (SEQ ID NO: 30) Del-1-proteins. The EGF-like and discoidin-like domains are indicated by "egf" and "discoidin," respectively.

5. On page 10, ll. 33-37, please delete the brief description for "Figure 8" and insert in its place the following:

Figure 8. The 54.2% amino acid homology between human Del-1 (SEQ ID NO:21) and MFG-E8 (SEQ ID NO: 20) in the tandem discoidin I/factor VIII domains is shown. These domains are rich in the basic amino acids arginine and lysine. The 5' domain contains 12 arginines and 12 lysines versus 9 acidic residues, while the 3' domain contains 8 arginines and 10 lysines versus 16 acidic residues. A similar domain in the coagulation factor VIII protein is believed to bind to negatively charged phospholipids on the surface of platelets. The MFG-E8 protein has been found to associate tightly with milk fat globule membranes.

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6. On page 12, ll. 4-6, please delete the brief description of "Figure 11" and insert in its place the following:

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Figure 11. Human del-1 splicing variant partial sequence (SEQ ID NO: 31) showing the variation as compared with the major form (SEQ ID NO:30).

7. On page 12, ll. 8-10, please delete the brief description of "Figure 12A-12E" and insert in its place the following:

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Figure 12A-12E.	Murine *del-1* truncated minor nucleotide and deduced amino acid sequences (SEQ ID NO: 28) and (SEQ ID NO: 29).

8. On page 44, ll. 6-15, please delete the paragraph and insert in its place the following:

The anti-angiogenic activity of Del-1 may be used to treat abnormal conditions that result from angiogenesis. These conditions include, but are not limited to, cancer, diabetic retinopathy, rheumatoid arthritis and endometriosis.

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Additionally, the removal or inhibition of Del-1 in situations where it naturally inhibits blood vessel formation may be used to promote angiogenesis. These conditions include, but are not limited to, cardiac ischemia, thrombotic stroke, wound healing and peripheral vascular disease. Furthermore, Del-1 may be used to stimulate bone formation.

9. On page 47, please delete the paragraph starting on l. 4 spilling over to page 48, l. 2, and insert in its place the following:

A 160 bp exon was trapped from a fragment of genomic DNA located approximately 10 kb from the "left" integration site. Nucleotide sequence of the

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trapped exon was employed to screen various nucleic acid databanks through the BLAST routine at the NCBI, revealing no other gene with significant nucleic acid homology. The deduced amino acid sequence of the single open reading frame was subsequently employed in databank searches. These revealed that the protein domain encoded in the trapped exon was similar in part to domains in a number of proteins, including Factor V, Factor VIII and discoidin I (Figure 2, SEQ ID NOS 1-8) (Jenny

et al., 1987, *Proc. Natl. Acad. Sci.* U.S.A. 84:4846-4850; Poole et al., 1981, *J. Mol. Biol.* 153:273-289; Toole et al., 1984, *Nature* 312:342-347) The protein which was most similar was milkfat globule protein, which had been found on the surface of mammary epithelial cells (1994, WO 94/11508). It has been hypothesized that the discoidin I-like domain in this protein allows it to localize to the surface of the epithelial cell (Larocca *et al.*, 1991, *Cancer Res.* 51:4994-4998; Stubbs *et al.*, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:8417-8421). The homologous regions of Factor V and Factor VIII have been implicated in their interaction with phospholipids on the surface of endothelial cells and platelets (Jenny *et al.*, 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:4846-4850; Toole *et al.*, 1984, *Nature* 312:342-347). Homology to the Xenopus protein A5 was also observed. A5 is a neuronal cell surface molecule which is expressed in retinal neurons and the neurons in the visual center with which the - retinal neurons contact (Takagi *et al.*, 1991, *Neuron* 7:295-307). A5 has been proposed to play a role as a neuronal recognition molecule in the development of this neural circuit, perhaps through mediating intercellular signaling. The protein for which this discoidin I-like domain was named is a protein expressed in *Dictyostelium discoideum*, which serves an essential role in the aggregation of individual cells.

10. On page 48, ll. 3-27, please delete the two paragraphs and insert the following in their place:

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The DNA fragment encoding the trapped exon was employed as a probe in a Southern blot experiment and shown to hybridize with regions of the *del-1* locus outside of the region that was employed in the exon trap construct. Given this

finding, cDNA cloning was pursued by using the exon trap probe to screen an 11.5 day embryonic mouse cDNA library. Clones were plaque purified, and inserts subcloned into plasmid for further analysis. Nucleotide sequence analysis showed that two of the embryonic cDNA clones contained the sequence of the trapped exon. Sequence from the clones was used to expand the deduced amino acid sequence of the discoidin I-like domain (Figure 2, SEQ ID NOS 1-8). The full nucleotide sequence of these cDNAs was analyzed and cloned into plasmid vectors which allowed the generation of cRNA transcripts for RNAse protection and *in situ* hybridization (Figure 3A-3D, SEQ ID NO: 9).

A human cDNA was isolated from a human fetal lung cDNA lambda phage library purchased from Clontech Inc. (Figure 4A-4C, SEQ ID NO: 11). A portion of the mouse *del-1* cDNA was used as a probe (Figure 5, SEQ ID NO: 19). The identity of the human cDNA clone was confirmed by comparing the human and mouse DNA sequences. These clones show approximately 80% DNA sequence homology and approximately 94% amino acid sequence homology (Figure 6, SEQ ID NOS: 10 and 30). These sequences are referred to as the "major" form of *del-1*. Upon initial isolation of *del-1*, standard molecular biology methods were used for isolating additional clones.

11. On page 48, please delete the paragraph starting from line 28 spilling over to page 49, line 3, and insert in its place the following:

DNA sequence analysis of the human *del-1* revealed an open reading frame of 1,446 base pairs predicted to encode a 481 amino acid protein with a molecular

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weight of, 53,797. The mouse cDNA encodes a 480 amino acid protein. Homology comparisons with DNA and protein databases indicated that the Del-1 protein was composed of three EGF-like protein domains, followed by two discoidin I/factor VIII-like domains (Figure 7). Genes similar to *del-1* included some key regulators of cell determination and differentiation such as Notch. Overall, the Del-1 protein has a structure similar to the membrane-associated milk fat globule membrane protein, MGF-E8, which has been used to develop antibodies for imaging breast cancer (Figure 8, SEQ ID NOS: 20 and 21).

12. On page 49, ll. 15-35, please delete the two paragraphs and insert in their places the following:

Key structural features of the open reading frame of human Del-1 include:

1) the presumed initiator methionine and putative secretion signal sequence (Figure 9, SEQ ID NO: 22)
2) the three EGF-like domains (Figure 10, SEQ ID NOS: 23-25)
3) the two discoidin I-like domains.

Further cloning and analysis of both the human and murine *del-1* genes revealed additional variant forms. For example, a human splicing variant (Z20 clone) was obtained in which 30 bp (*i.e.* 10 amino acids) (SEQ ID: 30 # 66-#75) between the first and second EGF-like domains of the major form (SEQ NO ID: 30) of *del-1* had been removed (Figure 11, SEQ ID NO: 31). In addition, a truncated version of murine *del-1* was isolated, which contained a signal peptide sequence, all three EGF-like domains and only a partial amino-terminal discoidin I/factor VIII-like domain (about 40%). This variant is referred to as murine *del-1* minor sequence, which is disclosed in Figure 12A-12D, SEQ ID NOS: 28 and 29. This transcript was cloned only from mouse embryonic libraries, but was verified through cloning of several independent cDNAs.

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13. On page 50, please delete the paragraph starting at line 23 spilling over to page 51, line 6 and insert in its place the following:

In order to study the expression of: the *del-1* gene, Northern blots containing RNA obtained from a variety of human and mouse tissues (Clontech, Palo Alto, CA) were hybridized with a radiolabeled DNA probe as shown in Figure 5, SEQ ID NO: 19. In addition, adult organs, 15.5 dpc whole embryos and organs dissected from embryos were disrupted with a polytron, and RNA isolated over CsCl gradient (Sambrook *et al.*, 1989, Molecular Cloning, Laboratory Manual, Cold Spring Harbor Laboratory, NY). Briefly, the blots were prehybridized at 42°C for 3-6 hours in a solution containing 5X SSPE, 10X Denhardt's solution, 100 μg/ml freshly denatured, sheared salmon sperm DNA, 50% formamide (freshly deionized), and 2% SDS. The radiolabeled probe was heat denatured and added to the prehybridization mix and allowed to hybridize at 42°C for 18-24 hours with constant shaking. The blots were rinsed in 2X SSC, 0.05% SDS several times at room temperature before being transferred to a wash solution containing 0.1X SSC, 0.1% SDS and agitated at 50°C for 40 minutes. The blots were then covered with plastic wrap, mounted on Whatman paper and exposed to x-ray film at -70°C using an intensifying screen.

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